

Influence of Probiotics on Microflora
in the Gastrointestinal and Reproductive Tracts of Quarter Horse Mares

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ABSTRACT

Current scientific literature is limited on what microorganisms are present in the equine gastrointestinal and reproductive tracts. Microbial populations in these environments can be easily disrupted and probiotics have been utilized in other species to re-establish homeostasis and inhibit the growth of pathogenic bacteria. The objective of this study was to determine whether probiotics could influence the microflora in the gastrointestinal and reproductive tracts of mature horses. Seven Quarter Horse mares (10 ± 2 yrs of age) were randomly assigned to 1 of 2 treatment groups (probiotic or control) for a period of 56 days. All horses received 0.5% BW of a 12% CP pelleted concentrate, with water and mixed grass hay *ad libitum*. Horses in the probiotic treatment group were fed a supplement containing *L. acidophilus* at a target dose of 10^9 cfu/45 kg of BW per day. Fecal samples and vaginal swabs were collected weekly to measure pH and evaluate changes in microflora. Uterine swabs were collected when possible during periods of estrus. Mean pH values were analyzed using the PROC MIXED procedure of SAS. A P value of ≤ 0.05 was considered statistically significant. There were no changes in fecal, vaginal or uterine pH due to probiotic supplementation. Microbial diversity was investigated using PCR with universal primers specific to 16S rRNA gene sequences and subsequent denaturing gradient gel electrophoresis (DGGE) analyses. Images were captured and analyzed with Bionumerics software to compare microbial diversity. PCR using universal primers was successful in amplifying the 200 bp region of interest in all samples. DGGE analysis of fecal and vaginal samples revealed that the control horses had a more diverse microflora compared to the horses given the probiotic. However, DGGE analysis of uterine samples revealed no differences in microbial populations due to the probiotic. The ability of a probiotic to colonize the host may be species and/or environment specific. Further analysis with *Lactobacillus*

specific primers is needed to determine the influence of the probiotic on microbial diversity within the gastrointestinal and reproductive tracts.

INTRODUCTION

Bacterial infections in the reproductive tracts of mares are one of the leading causes of infertility (Frontoso et al., 2008). Many times these bacterial infections are treated with antibiotics; however, the incorrect and overuse of antibiotics has led to a higher incidence of antibiotic resistance (Albihn et al., 2003). Therefore there is a need to find alternative methods to treatment to these infections. There is anecdotal evidence that probiotics can help with infertility in mares but there is no scientific data to support this.

Probiotics are live microorganisms that have proven beneficial to the host and help regulate a healthy immune response. In addition, they have been shown to be a safe alternative to antibiotics in helping prevent and treat urological infections (Fraga et al., 2008). The gastrointestinal tract functions as a barrier against antigens from microorganisms and food. The regulation of the gut depends on the establishment of indigenous microflora. Probiotics are believed to improve the gut's immunologic barrier and prevent pathogenic bacteria from invading and colonizing the gut, thereby creating a stabilizing effect. The use of probiotics as an alternative to antibiotics may be a less expensive treatment option but would also help reduce the risk of antibiotic resistance. This research will allow for new insights into manipulating microflora in the gastrointestinal and reproductive tracts of horses as well as potential alternatives to antibiotics in the treatment of uterine infections in the reproductive tracts of mares (LeBlanc., 2010).

MATERIALS AND METHODS

Seven Quarter Horse mares (10 ± 2 yrs of age) were randomly assigned to one of two treatment groups (Probiotic or Control) for a period of 56 days. All horses received 0.5% BW of a 12% CP pelleted concentrate, with water and mixed grass hay *ad libitum*. Horses in the probiotic treatment group were fed a supplement containing *L. acidophilus* at a target dose of 10^9 cfu/45 kg of BW per day. Fecal samples and vaginal swabs were collected weekly to measure pH and evaluate changes in microflora. Uterine swabs were collected when possible during periods of estrus. Mean pH values were analyzed using the PROC MIXED procedure of SAS. A P value of ≤ 0.05 was considered statistically significant.

Immediately after collection, uterine and vaginal swabs were placed in 10 mL of LB broth and incubated at 37°C for 24 h. DNA extraction and purification of the uterine and vaginal samples was done using a Qiagen Mini DNA kit (Qiagen, Inc., Valencia, California). Fecal samples were collected and stored at -80° C until further analysis. Microflora assessment of the fecal samples was determined via Repeated Bead Beating Plus Column RB++C (Yu and Morrison, 2004) with a modified protocol for elution of adding 50 μ L instead of 200 μ L of AE. DNA extraction and purification was done using a Qiagen Mini DNA kit (Qiagen Inc., Valencia, CA). Purified DNA was run on a 0.8% agarose gel at 100v for 1h. Quantification of DNA was determined via a nanodrop (NanoDrop Technologies, Montchanin, DE). DGGE-PCR was analyzed on DNA purified samples (Yu and Morrison, 2004). The primers used to derive 16S rDNA-targeted primers for amplification of all bacterial species were HDA1 (5'-3'): AC TCC TAC GGG AGG CAG CAG and HDA2 (5'-3'): GTA TTA CCG CGG CTG CTG CGA (Walter et al., 2000). A 40 bp GC clamp was attached to the reverse primer Lac2 to obtain PCR product suitable for DGGE. A 39 bp GC clamp was attached to the forward universal primer. The

melting and annealing temperature for specific primers were determined and validated by Yu and Morrison (2004). The reaction mixture (50 μ L) contained 0.255 μ L of each 100 uM primer and Taq polymerase, 1 μ L of the DNA template, 1.02 μ L of BSA, and 3.57 μ L of 50 mM $MgCl_2$, and 0.408 μ L dNTP.

Prior to DGGE, 5 μ L of each PCR product were subjected to 2% agarose gel electrophoresis to confirm successful amplification of the V3 region. Then, 10 μ L aliquots of PCR product were resolved in a 7.5% polyacrylamide gel containing a 40%-60% gradient of denaturants (formamide and urea). The DGGE gel was run in 1% TAE at 60° C and 82 V for 16 h using INGENY phorU-2 (Ingeny; Leiden, The Netherlands) and the images were captured using a FluorChem® Imager (Alpha Innotech, San Leandro CA). Images were then analyzed with a software program to compare bands across gels (Bionumerics; Applied Maths, Austin, TX).

RESULTS

Throughout the study, there were no changes in fecal ($P = 0.65$) or vaginal ($P = 0.28$) pH due to probiotic supplementation (Figures 1 and 2, respectively). PCR using universal primers was successful in amplifying the 200 bp region of interest in all samples. DGGE analysis of fecal (Figure 3) and vaginal (Figure 4) samples revealed that the control horses had a more diverse microflora compared to the horses given the probiotic. However, DGGE analysis of uterine samples revealed no differences in microbial populations due to the probiotic (data not shown).

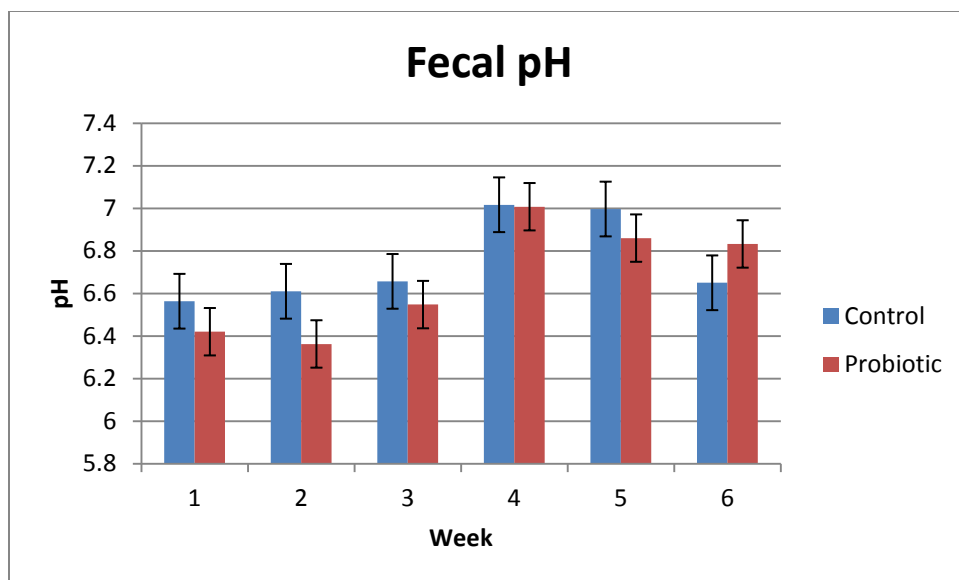


Figure 1. Effect of probiotic supplementation on fecal pH in Quarter Horse mares.

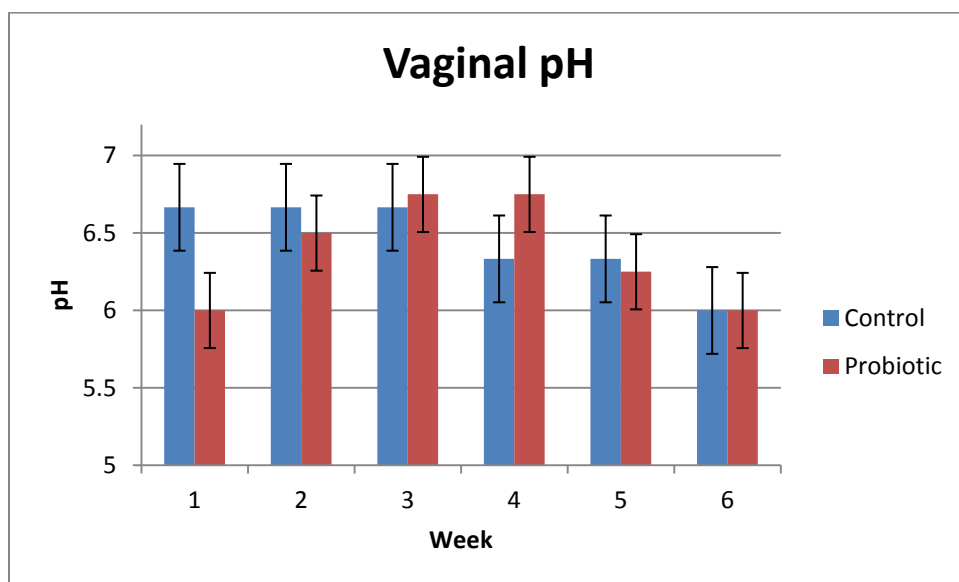


Figure 2. Effect of probiotic supplementation on vaginal pH in Quarter Horse mares.

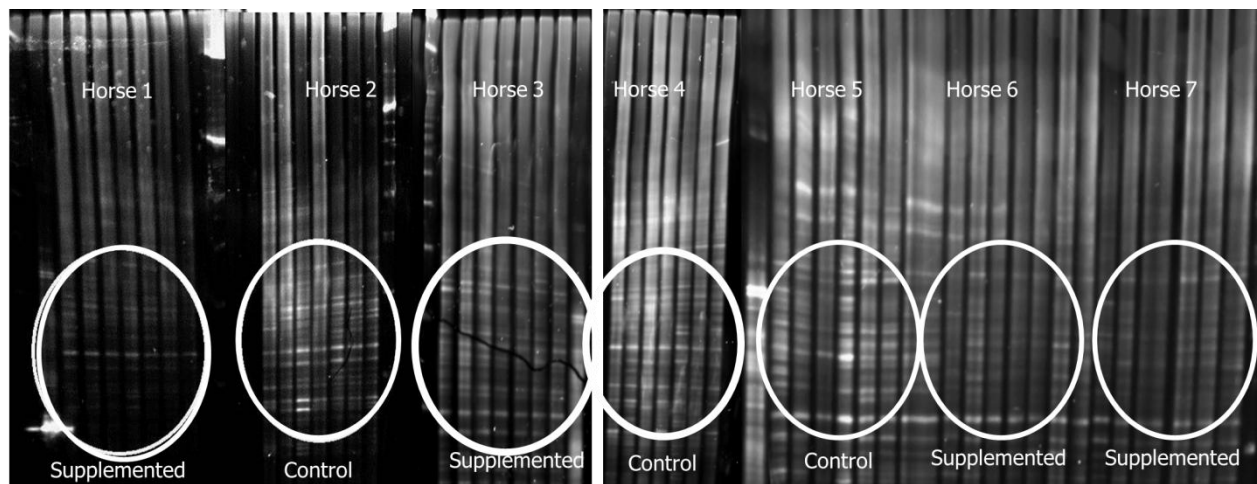


Figure 3. Effect of probiotic supplementation on microbial profiles in the gastrointestinal tract of Quarter Horse mares shown through DGGE.

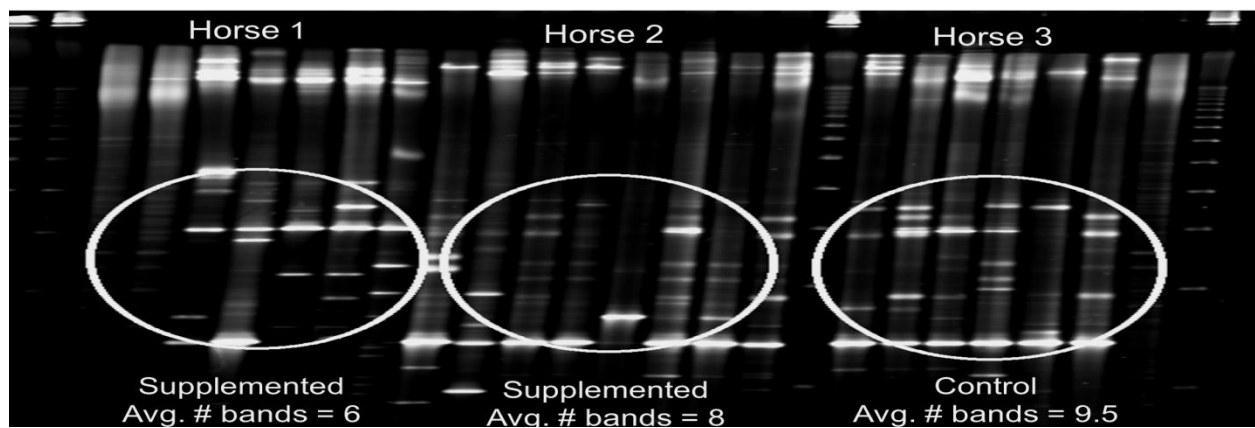


Figure 4. Effect of probiotic supplementation on microbial profiles in the gastrointestinal tract of Quarter Horses as shown through DGGE.

DISCUSSION

The effects of probiotic supplementation on the gastrointestinal microflora of horses has been evaluated with varying results (Weese et al., 2002; Weese and Rosseau, 2005; Parraga et al. 2008). However, this is one of the first studies to evaluate the influence of probiotics on the microflora in the reproductive tract of horses. In the present study, mares that were fed the probiotic supplement had a less diverse microflora in the gastrointestinal tract compared to the control horses. This suggests that the *Lactobacillus acidophilus* were able to colonize the gastrointestinal tracts of the mares and potentially exclude other bacterial species but further analysis with *Lactobacillus* specific primers is needed to support this hypothesis.

Although DGGE analysis of vaginal samples revealed that the mares given the probiotic supplement had less microbial diversity than the control horses, the same analysis of uterine samples showed no differences in microbial populations in response to the probiotic supplement. The ability of probiotics to colonize the host may be dependent on the bacterial species and the environment itself (Weese, 2002). Additional studies are needed to evaluate factors that influence the microbial diversity in the gastrointestinal and reproductive tracts of horses to determine if the use of probiotics is a feasible alternative to antibiotics in the treatment of uterine infections.

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